

CLAIMS

1. A method for increasing the number of polynucleotides containing sequences corresponding to a mRNA species present in a sample, the method comprising the steps of:
 - 5 (i) reverse transcription of the mRNA species using a heeled 5'-amplification primer (FAP-RAND) and a heeled 3'-amplification primer (TAP-RT), wherein each primer sequence is unique, and either or each heel sequence includes a RNA polymerase promoter site, and the FAP includes a variable sequence, whereby the RNA is reverse-transcribed to produce double-
10 stranded cDNA and then multiple cDNAs according to the variable sequence; and
 - (ii) amplification of the cDNA using primers sufficiently complementary to the primers, i.e. FAP and TAP, within FAP-RAND and TAP-RT.
2. A method according to claim 1, which additionally comprises the step of:
 - 15 (iii) *in vitro* transcription, to produce RNA run-offs from either end of the amplicons.
3. A method according to claim 1 or claim 2, wherein each heel sequence includes a different RNA polymerase site.
4. A method according to claim 3, for the production of a strand-specific library.
- 20 5. A method according to any preceding claim, for the production of a subtracted library from two cell populations.
6. A method according to any preceding claim, which comprises cloning the polynucleotide products and immobilising them in an array.
7. A method according to any preceding claim, wherein the sample is from laser
25 capture microdissection.
8. A method according to any preceding claim, wherein the sample is from patch clamp harvesting.
9. A method according to any preceding claim, wherein the first and/or the second heel sequence includes the nucleotide sequence of a cleavage site.
- 30 10. A method according to claim 9, wherein the cleavage site is located at the 3' end of its heel sequence.
11. A method according to claim 10, wherein the first and second heeled primers have identical cleavage sites.

12. A method according to claim 10, wherein the first and second heeled primers have different cleavage sites.
13. A method according to any of claims 9 to 12, which comprises the additional step of treating the polynucleotides with an agent that cleaves at the cleavage site.
- 5 14. A method according to any preceding claim, wherein amplification comprises up to 50 amplification cycles.
15. A method according to claim 14, wherein each amplification cycle comprises the steps of :
- (i) obtaining single-stranded DNA molecules at between 85°C and 97°C;
 - 10 (ii) annealing the single-stranded DNA molecules at between 45°C and 65°C;
- and
- (iii) elongating the annealed DNA molecules at between 70°C and 75°C .
16. A method according to any preceding claim, wherein the first heeled primer population consists of a population of nucleic acids comprising, from 5' end to 3' end :
- 15 (i) a heel sequence, of 15 to 22 nucleotides, which is not complementary to the mRNA molecules initially present in the sample; and
- (ii) an oligo dT sequence of 15 to 25 nucleotides;
- wherein substantially every possible variable sequence combination is found in said first heeled primer population.
- 20 17. A method according to any preceding claim, which additionally comprises confirming the presence of at least one nucleic acid sequence contained in the reaction mixture after amplification.
18. A method according to claim 17, wherein the confirming comprises any one of:
- (i) detection of sequences of interest with specific oligonucleotide probes;
 - 25 (ii) amplification of sequences of interest with specific oligonucleotide primers;
- and
- (iii) cloning of the DNA molecules obtained in a replication and/or expression vector.